Effect of selected plant biostimulants and calcium on the performance of *Foeniculum vulgare* Mill.

Synopsis

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PARNIKA JINDAL

Under the supervision of

DR. AKBAR ALI

Department of Botany, Faculty of Science
Dayalbagh Educational Institute (Deemed to be University)
Dayalbagh, Agra – 282 005

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Introduction

India has always been recognized as a “Land of Spices” or "Home of Spices" in which the seed spices constitute an important group of agricultural commodities and play a significant role in national economy. *Foeniculum vulgare* Mill. (Fennel) is commonly known as “saunf” which is a cuisine spice that belongs to the Apiaceae family. India is the world's largest fennel producing country with Syria, Egypt, Turkey, Germany, Spain and Pakistan also making significant contributions to global production. India and Egypt are significant exporters of fennel (ICAR, 2021). In India, it is mainly cultivated in Gujarat, Rajasthan, Madhya Pradesh, West Bengal and Uttar Pradesh. Fennel is cultivated on 79842 hectares of land in India, with a total production of 128497 tonnes and an average productivity of 1609 kg ha^{-1} (Shivran et al. 2023). Gujarat and Rajasthan are major fennel producing states, contribute about more than 90 percent of the total fennel production in the country (Shivran et al. 2023). Due to its commercial significance and considerable pharmaceutical uses, fennel is regarded as one of the world’s most important medicinal plants while being one of the oldest spice plants (Noreen et al. 2023). Out of the 422,000 flowering plants that exist worldwide, more than 5000 are used medicinally (Noreen et al. 2023). Medicinal plants possess different kinds of valuable metabolites such as essential oil, antioxidants and flavonoids. These are the complex secondary metabolites which are derivatives of terpenes, terpanoids and phenyl perpanoids (Angane et al. 2022). The fruits, foliage, as well as bulbs of fennel, are used medicinally and as vegetables worldwide (El-Beltagi et al. 2023). Essential oil (EO) is a mixture of heterogeneous organic, aromatic and volatile liquid obtained from bark, flower, bud, rhizomes, root, leaves, stem, wood, and even from whole plant (Brenes & Roura, 2010; Angane et al. 2022). Fennel Essential oil is extracted from its stem (0.21%), leaves (0.83-1.5%) and seeds (0.6-6%) (Malhotra et al. 2012; Özel et al. 2019; Šunić et al. 2023). But the economic value of this crop is related mainly to the seeds which contains 70 to 90 percent of fennel essential oil (Saharkhiz and Tarakeme, 2011). The major active constituents of fennel essential oil are anethole and fenchone. The liquorice like flavour of fennel is due to the presence of active constituent anethole (Mehra et al. 2021). Anethole is known as a flavoring agent in the food industry and perfume industry, as well as an anti-bloating compound in traditional medicine and an effective substance in the taste and smell of fennel (Sabzi-Nojadeh et al. 2021).

Plant biostimulant

Chemical fertilizers are being used in agriculture at an alarmingly rapid rate, endangering both the environment and public health. The dependence on chemical fertilizers in the agriculture industry can be decreased through biostimulants (Du Jardin, 2015). They may lessen stress, brought on by climate change and restrict eutrophication, without endangering the environment. A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, stress tolerance, yield quantity and quality (Du Jardin, 2015), for example seaweed extracts, plant extracts, humic and fulvic acid, melatonin, trehalose, etc. have been used in commercial agriculture for decades, but in more recent years the number of these products available to and used by growers has markedly increased. The global market for these products was estimated at USD 2.6 billion in 2019 with a projected value in 2025 at over USD 4 billion (Sible et al. 2021).

Melatonin (MEL)

MEL is chemically known as N-acetyl-5-methoxytryptamine **(**$C_{13}H_{16}N_2O_2$**),** is an amphiphilic low molecular weight indoleamine (Lerner et al. 1958), and is a phytohormone that has recently gained attention as a potential biostimulator in agriculture. It has been shown to have numerous beneficial effects on plant growth and development, as well as on the ability of plants to tolerate abiotic stress (Khan et al. 2023a). It may easily diffuse into the cytosol, nucleus, and mitochondria after passing through the cell membrane (Debnath et al. 2019). Foliar application of MEL increases photosynthetic efficiency and decrease photoinhibition, which leads to higher biomass accumulation and yield (Ramírez-Estrada et al. 2023).

Trehalose (Tre)

Sugars play crucial roles in a wide range of metabolic and developmental activities throughout the whole plant life cycle, that is, from the juvenile stage to the reproductive stage. Trehalose (α-D-Glucopyranosyl-1, 1-α-D-glucopyranoside; $C_{12}H_{22}O_{11}$) is a non-reducing disaccharide formed through an α, α - 1, 1-glycosidic bond linking two glucose moieties. It is a naturally occurring sugar that may be found in a variety of living organism, including bacteria, fungi, plants, insects, and other invertebrates (Kosar et al. 2019; Shi et al. 2019; Ambrosio' et al. 2020). Tre is an osmoprotectant and acts as a membrane/molecule stabilizer. It stabilizes dehydrated proteins, enzymes, lipids as well as

biological structures from damage/injures during desiccation (Islam and Mohammad, 2021) and is effective in regulating various physio-biochemical processes in plants, which may have a direct or indirect role in stimulating plant growth and yield production.

Calcium (Ca)

Foliar nutrient application is faster than traditional soil application, enables quick translocation of nutrients and speeds up the transport of minerals via the stomata, although total absorption may be higher through the epidermis (Kant et al. 2023). There are twenty elements to be essential for plant that are classified as macro nutrient viz nitrogen, phosphorus, potassium, calcium, sulphur, magnesium, carbon, oxygen and hydrogen micronutrient viz iron, boron, chlorine, manganese, zinc, copper, molybdenum, nickel, cobalt, sodium, silicon. Ca is one of the macro nutrients that has many physiological functions in the growth and development of the plants and acts as secondary messenger molecule in plants (Kader and Lindberg, 2010). It influences intake of nitrogen, promotes early root formation and growth (Sajid et al. 2020). Ca controls the rigidity of the cell wall, thus, the middle lamella of the pectin is cross-linked by Ca between negatively charged carboxyl groups. Through its interactions with phospholipids, Ca not only strengthens cell walls but also maintains the stability of cell membranes (Thor, 2019). It also plays the role in maintaining the metabolism of carbohydrates, protein, meristematic activity, cell division, and cell elongation (Majeed and Bayati, 2023).

Need of this study-

- There is a growing demand for fennel essential oil in the market, increasing production can help meet this demand and prevent supply shortages.
- Price of fennel essential oil is high (Rs. 2200 to 6500 per kg).
- Fennel has huge need in industries such as in pharma sectors, perfumery industry, culinary, and confectionary.
- Fennel essential oil is a value-added product derived from fennel seeds. Increasing production can provide additional income for farmers and stakeholders involved in the fennel cultivation and essential oil extraction practices.
- Fennel essential oil is not only used domestically but also exported globally. Expanding production can help capture these export opportunities and contribute to the country's economy.

- To the best of my knowledge, there is lack of information on the foliar application of plant biostimulants and calcium on *Foeniculum vulgare* Mill.

Therefore, keeping the above facts in view, the goal of this study is to improve growth and yield of fennel plants using selected plant biostimulants and calcium which will be environment friendly and cost effective and findings of this study will facilitate us to understand the mechanism of melatonin, trehalose and calcium.

Objectives-

1. To study the effect of foliar application of melatonin on growth, physiological, biochemical and yield parameters of *Foeniculum vulgare* Mill.
2. To evaluate the role of foliar application of trehalose on growth, physiological, biochemical and yield parameters of *Foeniculum vulgare* Mill.
3. To investigate the combined effect of best dose of melatonin with different concentrations of calcium on growth, physiological, biochemical and yield parameters of *Foeniculum vulgare* Mill.
4. To investigate the combined effect of best dose of trehalose with different concentrations of calcium on growth, physiological, biochemical and yield parameters of *Foeniculum vulgare* Mill.

Review of literature

Systematic position of the plant

According to the system of classification by Arthur Cronquist (1981), *Foeniculum vulgare* Mill. occupies following systematic position-

Table 1 CLASSIFICATION OF FENNEL

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Apiales
Family	Apiaceae
Genus	*Foeniculum*
Species	*Vulgare*

Figure 1 *Foeniculum vulgare* Mill.

Description of the plant

Foeniculum vulgare Mill. is a tall, erect, glabrous, glaucous, biennial or perennial herb, with yellow flowers, aromatic seeds and compound leaves. The leaves grow up to 40 cm long stalked with a long petiole; they are finely dissected with the ultimate segments filiform (thread like) of about 0.5 mm wide. Flowers are bisexual, actinomorphic and open centripetally. The fruit color is light green to dark brown, lens-shaped schizocarp, oblong-oval to elliptical 2-3 mm with long pedicel and short stylopodium. The flowers are produced in terminal compound umbels. A fully developed fruit is about 4 to 8 mm long. Fennel seeds are elliptical, slightly curved, and somewhat obtuse at the ends (Badgujar et al. 2014).

Varieties of *Foeniculum vulgare* Mill.-

There are commonly three fennel varieties cultivated in India - *Foeniculum vulgare* Mill. var. vulgare, *Foeniculum vulgare* Mill. var. dulce and *Foeniculum vulgare* Mill. var. azoricum (Jadid et al. 2023).

Cultivars of *Foeniculum vulgare* Mill.-

Cultivars of *Foeniculum vulgare* are AF-1, AF-2, GF-1, GF-2, GF-11, PF-35, Hisar Swarup, Co-1 (Malhotra and Vashishtha, 2008), RF-101, RF-125, RF-143, RF-145, RF-178, RF-205, RF-290, RF-157, RF-281, RF-289 (developed by SKNAU, Jobner, Rajasthan), CIM-Sujal (developed by CSIR-CIMAP, Lucknow).

Chemical constituents of *Foeniculum vulgare* Mill.-

Essential oil is extracted from its roots, stem, leaves and seed parts of fennel. Essential oil obtained from seeds contains 70 to 90 percent of fennel essential oil with oil content of 0.6 to 6%. Trans-Anethole (14.84-86.1%), Fenchone (0.54-15.8%), Alpha-Pinene (2.09-15.8%), Beta-Pinene (2.23-32.82%), MethylChavicol (2.7-15.33%), Myristicin, Limonene, Exo-FenchylAcetate, Gamma-Terpinene, Myrcene, Alpha-Phellandrene, Alpha-Bergamotene, P-Cymene, Alpha-Copaene, Bornyl Acetate, Delta-Cadinene, Elemicine, Eugenol, Methyl Eugenol are all active constituents of fennel seeds. Fruits of fennel contain iodine, vitamin A, thiamine, riboflavin, niacin and ascorbic acid in minute quantities also contains significant amount of calcium, phosphorous and potassium (Hashmi et al. 2012).

Industrial uses

Fennel is used in many industries such as in food, perfumery, and confectionery industries. The leaves and seeds of fennel are used in many culinary preparations (Ehsanipour et al. 2012). Mature fennel seeds and essential oil are used as flavouring agents such as in liqueurs, bread, pickles, pastries and cheese (Zoubiri et al. 2014). Fennel seeds are consumed as *mukhwas* (Mouth freshener) and also as fennel tea.

Pharmacological activities

Fennel has various pharmacological and therapeutic properties including anti-allergic, analgesic, anti-inflammatory, antioxidant, antibacterial, anti-cancer, anti-spasmodic, anti-stress, and cytotoxicity activity (Noreen et al. 2023). Due to the plant's diuretic qualities, it is used to treat kidney and bladder diseases. Additionally, it is utilised to reduce nausea and halt vomiting. The herbs can be used to alleviate blockages in the hepatic, gastrointestinal, respiratory, and urinary systems in addition to persistent fever. Additionally, they are used to address issues with the eyes, such as cataracts (Bettaieb Rebey et al. 2019; Noreen et al. 2023).

Melatonin

Khan et al. (2023b) reported that the foliar application of melatonin (50 or 100 µM)) increased photosynthesis, enhanced cell viability, stomatal behavior, proline content, activity of catalase (CAT), peroxidase (POX), and superoxide dismutase (SOD) while decrease in malondialdehyde (MDA) content in tomato plants under stressed and non-stressed conditions.

Gholami et al. (2022) found that foliar treatment with melatonin could combat the negative effect of drought, by preserving the composition of fatty acids in membrane lipids, increasing the synthesis of antioxidant protective molecules that also improved the nutraceutical and nutritional value of olive oils, stabilising oil yield, and preserving oil quality and yield.

Naghizadeh et al. (2022) showed that foliar application of melatonin on *Plantago ovata* enhanced the growth and yield parameters such as number of tillers per plant, number of spikes per plant, spike length, number of seeds per spike, and 1000-seed weight and grain yield. Hence, melatonin increases the quantitative and qualitative yield of the seeds.

Ahmad et al. (2020) showed the effect of the foliar application of melatonin at a concentration 50 µM

during the process of leaf senescence, increased the activities of antioxidant enzymes, which in turn greatly decreased the malondialdehyde (MDA) content and reactive oxygen species (ROS) accumulation. The grain production of maize crop in semi-arid location was considerably greater with plants treated with melatonin due to improved photosynthetic activity, antioxidant defence system, and decreased leaf senescence.

Mir et al. (2020) concluded that melatonin stimulates *Brassica juncea* development through influencing the photosynthetic and antioxidant systems. The increased CO_2 inflow into the leaf cells is caused by melatonin which also increases stomatal conductance and opening.

Xiao et al. (2019) studied that low concentrations of melatonin promoted seed germination, and displayed, the best germination occurring after treatment with 20 μM melatonin, which also markedly increased GA3 content and decreased ABA content.

Trehalose

Fan et al. (2023) found that the fruit quality of cherry tomatoes was significantly affected by treatment solutions containing 0.5 and 1% trehalose. Cherry tomatoes' ability to retain, antioxidant enzyme activity, was most significantly impacted by a 0.5% trehalose solution. Tre may therefore be useful in minimizing deterioration and maintaining the quality of cherry tomatoes**.**

Al-Khafaji and Al-jubouri, (2022) studied the foliar application of trehalose, exhibited high vegetative and production traits in the carrot plant than those that did not receive foliar trehalose treatment. Additionally, trehalose spray helps in nitrogen assimilation in leaves.

Li et al. (2022) concluded that exogenous application of trehalose increased photosynthetic capacity, dry matter (root and shoot) accumulation, carbon and nitrogen metabolic activity in maize.

Islam and Mohammad, (2021) demonstrated that foliar application of 10 mM Tre (Trehalose) significantly improved the performance of Indian mustard. This treatment effectively increased the accumulation of osmolytes and enzyme activity, leading to a reduction in ROS chemicals. Consequently, this resulted in higher levels of photosynthetic pigments, gas exchange, mineral acquisition, root cell viability, and overall physio-biochemical parameters. These positive changes ultimately enhanced the biomass, yield, and quality of Indian mustard crops.

Habib et al. (2020) indicated that Trehalose application @ of 20 mg/L showed significant

improvements in physiological and biochemical attributes of *Ocimum tenuiflorum*, with noticeable enhancement in phenol content, suggesting an elevated defense mechanism in the plant.

Calcium

Ali et al. (2023) reported that the foliar application of calcium and chitosan enhanced growth indicators, such as the number of leaves, chlorophyll content in the leaves, dry matter and carbohydrates in the leaves, as well as the percentage of calcium.

Kader, (2023) demonstrated, the enhancement in vegetative properties, nitrogen, phosphorus, potassium, calcium, chlorophyll and leaf area, fruit size and fruit chemical quality due to foliar application of calcium in *Punica granatum* L.

Khalaj et al. (2023) reported that in gladiolus, foliar treatment with calcium nitrate (1.5 g L^{-1}) enhanced flowering parameters such as flower length, diameter, vase life and other growth parameters.

Majeed and Bayati, (2023) studied that the application of Ca improved the fruit quality content. Additionally, this research also showed that tomato sprayed with Ca has better vitamin C content in tomato fruits.

Morales-Sillero et al. (2021) studied the effect of foliar application of $CaCl_2$ (0.5% and 1.0%) on different stages of fruit development. Foliar treatment of calcium enhanced the amount of hydroxytyrosol, tyrosol, and oleuropein, among other phenols, and the oil content in the pulp (dry weight basis).

Proposed Methodology

Experiment site-

The experiments will be conducted at Agriculture farm, Dayalbagh Educational Institute, Dayalbagh Agra, Uttar Pradesh, India.

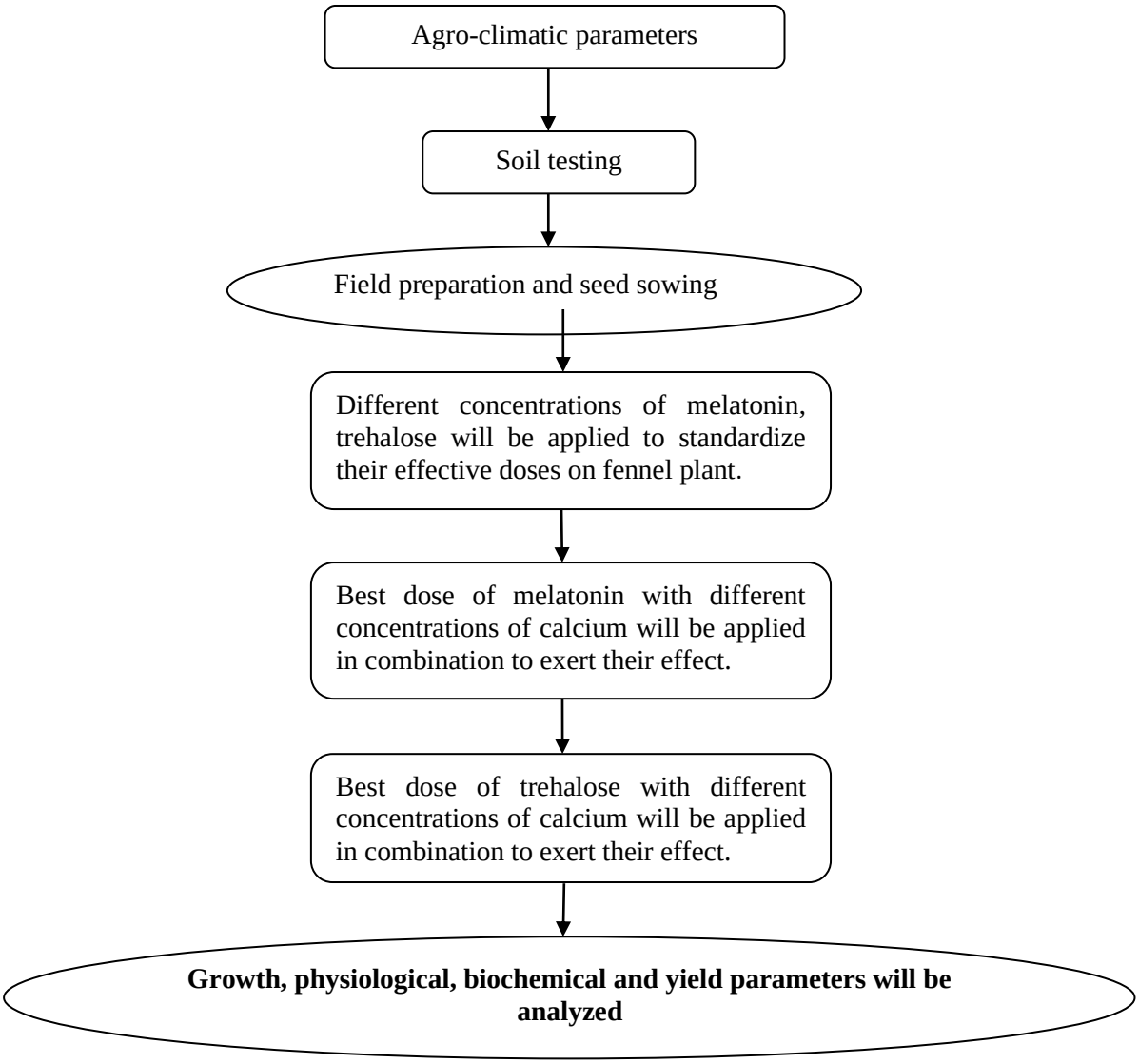
Agro-climatic parameters
Soil testing
Field preparation and seed sowing
Different concentrations of melatonin, trehalose will be applied to standardize their effective doses on fennel plant.
Best dose of melatonin with different concentrations of calcium will be applied in combination to exert their effect.
Best dose of trehalose with different concentrations of calcium will be applied in combination to exert their effect.
Growth, physiological, biochemical and yield parameters will be analyzed

Figure 2 Experimental Work Plan

After completion of all treatments, the following parameters will be analyzed-

1. Growth parameters -

- Shoot length per plant
- Root length per plant
- Fresh weight of shoot per plant
- Dry weight of shoot per plant
- Fresh weight of root per plant
- Dry weight of root per plant
- Number of branches per plant

- Number of umbels per plant
- Number of umbellates/umbel/plant

2. Physiological and biochemical Parameters –

- Total chlorophyll content (Maclachlan and Zalik, 1963)
- Total carotenoids content (Maclachlan and Zalik, 1963)
- Leaf-nitrogen content (Kjeldhal method/Lindner, 1944)
- Leaf-phosphorus content (Spectrophotometer/Fiske and Subbarow, 1925)
- Leaf-potassium content (Flame photometer/Hald, 1947)
- Calcium content (Flame photometer)
- Photosynthetic rate by IRGA (Infrared Gas Analyzer)
- Stomatal conductance by IRGA (Infrared Gas Analyzer)
- Transpiration rate by IRGA (Infrared Gas Analyzer)
- Nitrate reductase activity (NRA) (Jaworski, 1971)
- Carbonic anhydrase activity (CA) (Dwivedi and Randhwa,1974)
- Seed carbohydrate content (Hedge et al. 1962)

3. The yield Parameters

Essential oil extracted from seeds by hydro-distillation method using Clevenger's apparatus and then quantified gravimetrically according to Guenther (1972).

- Weight of umbels per plant
- Weight of 1000 seeds
- Seed yield per plant
- Essential oil content per plant
- Essential oil yield per plant

Oil content will be estimated by the following formula:

$$\text{Oil } (\%\frac{v}{w}) = \frac{\text{Observed volume of oil}}{\text{Weight of sample}} \times 100$$

Active constituents will be analyzing by using Gas Chromatography (GC) /Gas Chromatography-Mass Spectroscopy (GC-MS).

- Trans-Anethole content per plant
- Trans-Anethole yield per plant
- Fenchone content per plant
- Fenchone yield per plant

Statistical analysis

Statistical analysis will be done by statistical software.

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